The Role of Endoglin in Insulin-Mediated Angiogenesis

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INTRODUCTION

Angiogenesis is essential for tumor survival and progression in that it provides a blood supply for the tumor to increase in size, and TGF-β Smad-dependent signaling is critical in regulating this process. Endoglin, a transmembrane coreceptor of the TGF-β pathway, is expressed in endothelial cells including those of the vasculature, and thus contributes to regulation of pro- and anti-angiogenic processes. Recently, our lab discovered novel cross-talk between the insulin and TGF-β pathways. The purpose of this study is to examine the role of endoglin in insulin-mediated Smad signaling. Phosphorylated Smad1/5/8 is a pro-angiogenic transcription factor that cancer tumors need to grow. Immunofluorescence and biochemical methods were used to see cellular changes and differences in protein concentrations. Preliminary data shows that insulin treated cells that contain endoglin have a higher concentration of phosphorylated Smad1/5/8.

HYPOTHESIS

The purpose of this study is to examine whether endoglin in insulin-mediated Smad signaling activates phosphorylation of Smad1/5/8, a proangiogenic transcription factor.

METHODS

Western blotting was used to determine relative amounts of endoglin under various conditions. Immunofluorescence was used to directly visualize endoglin and insulin in the cell and whether they are overlapping, indicating interaction between the two proteins. Immunoprecipitation was used to see whether insulin receptor was interacting with endolgin by immunoprecipitating endoglin and blotting for insulin receptor. The blot reveals whether insulin receptor was attached to endoglin *in vitro*.

Overview of TGF-β Signaling

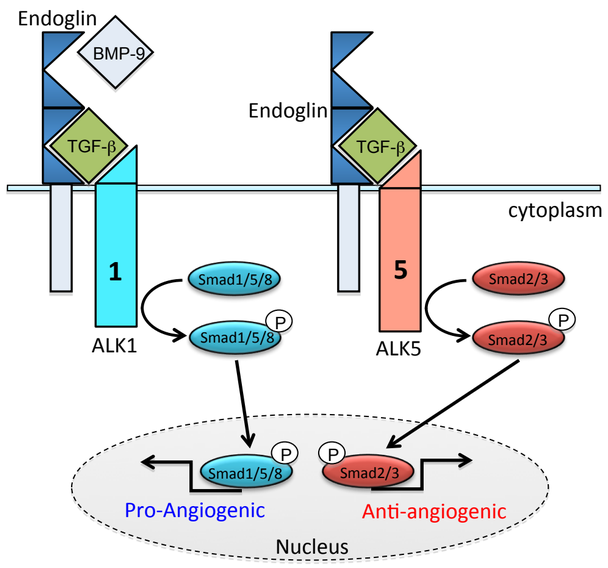

Fig 1. Balance between Smad1/5/8 and Smad2/3 is critical in regulation of cellular processes.

RESULTS

Insulin Increases Smad 1/5/8 Phosphorylation

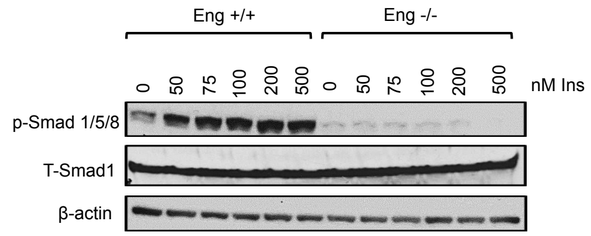

Fig 2. Western blot showing the induction of p-Smad 1/5/8 in the presence of endoglin due to the insulin treatment, indicating that endoglin is required for insulin to stimulate Smad 1/5/8 activation.

Endoglin and Insulin Receptor Co-localize

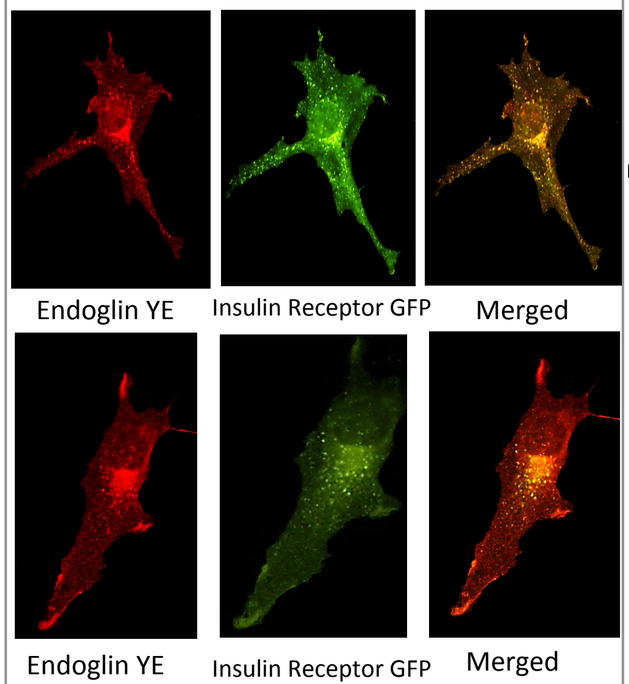

Fig. 3: Immunofluorescence labeling of endolgin (red) and insulin receptor (green) in endothelial cells demonstrates co-localization *in vitro* (yellow).

Insulin Receptor and Endoglin Directly Interact

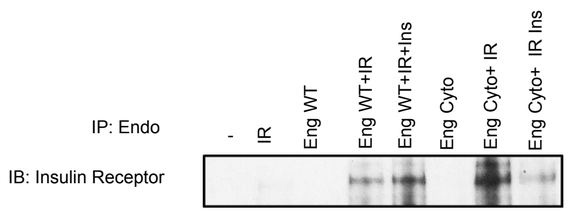

Fig 4. Blot showing immunoprecipitatation of endoglin and insulin receptor from endothelial cells supports that insulin receptor is directly interacting with endoglin *in vitro*.

ALK1 is Necessary for Insulin-Dependent Smad 1/5/8 Activation

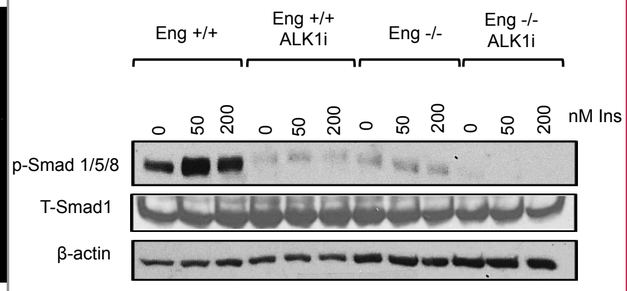

Fig 5: Western blotting reveals that ALK1 inhibition reduces *in vitro* activation of Smad 1/5/8 upon insulin stimulation in endoglin positive endothelial cells, indicating that endoglin and ALK1 are needed for insulin-induced Smad 1/5/8 activation.

CONCLUSIONS

- Insulin treated cells that contain endoglin have a higher concentration of phosphorylated Smad 1/5/8 compared to endoglin null cells.
- Given that type 2 diabetic patients are susceptible to a number of vascular-related conditions and malignancies, our results reveal new pathophysiologic implications for Smad1/5/8 signaling through hyperinsulinemia during pre-diabetic and diabetic disease progression.
- The findings of this study may provide information to better understand how type 2 diabetic patients develop vascular abnormalities and cancer.